IPD Project Details

Project ID: IPD8910

Project Title: A wolf in another wolfâ€™s clothing: Post-genomic regulation dictates venom profiles of medically-important cryptic kraits in India

Description: This study investigates the widespread cryptic Bungarus caeruleus (Comman krait) in India and clinical repercussions of their bits by implementing multidisciplinary approach involving proteomics, transcripomics, and assessment of toxicity and efficacy of currently marketed anivenoms. The study also aims at understanding the evolutionary relationships between these phenotypically simillar kraits.

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Sample Preparation: Crude snake venom samples (50 µg) in 25 mM ammonium bicarbonate were reduced with 10 mM dithiothreitol for 30 min at 37°C, alkylated with 100 mM iodoacetamide for 30 min in the dark and digested with trypsin (0.2 µg/µL) at 37° C overnight, in a total volume of 50 µL. The reaction was stopped using 0.1% formic acid, and desalting was carried out to remove the buffer salt and detergent using ZipTip with acetonitrile.

Peptide Separation: The resulting tryptic peptide mixtures were separated using Thermo EASY nLC 1200 series system (Thermo Fisher Scientific, MA, USA) coupled online with a Thermo Orbitrap FusionTM Mass Spectrometer (Thermo Fisher Scientific, MA, USA). 0.5 µg of peptide mixture was loaded on a 50 cm x 75 µm C18 (3 µm, 100 Å) nano-LC column. The mobile phase solution consisted of 0.1% formic acid in HPLC grade water (solution A) and elution buffer of 0.1% formic acid in 80% acetonitrile (solution B). The flow rate of solution B was set to 300 nL/min and was used in the following concentrations: 10-45% over 98 min, 45-95% over 4 min and 95% over 18 min.

Protein Characterization: MS spectra were acquired in positive mode at 2.0 kV, with a capillary temperature of 200°C, using 1? scan in the range 375-1700 m/z, maximum injection time of 50 ms and resolution of 120,000. Fragment scans (MS/MS) were performed using an ion trap detector with high collision energy fragmentation (30%),

scan range between 100-2000 m/z and maximum injection time of 35 ms. For the identification of various toxin families in the proteomic profiles of venoms, raw MS/MS spectra were searched against the National Center for Biotechnology Information's (NCBI) non-redundant (nr) database (Serpentes: 8570; November 2020), as well as the venom gland transcriptomes generated in this study, using PEAKS Studio X Plus (Bioinformatics Solutions Inc., ON, Canada). Parent and fragment mass error tolerances were set at 10 parts per million (ppm) and 0.6 Da, respectively. Cysteine carbamidomethylation was set as fixed modification while methionine oxidation and deamidation of asparagine or glutamine were set as variable modifications. A maximum of 2 missed cleavages by trypsin in the semispecific mode were allowed. Filtration parameters for match acceptance were set to FDR 0.1%, detection of ?1 unique peptide and -10lgP protein score ?40. Hits with at least one unique matching peptide were considered for downstream analyses.

Experiment Type: Shotgun proteomics

Species: Bungarus-8612

Tissue: Venom (bto:0001439)

Cell Type:

Disease: Unknown

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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